ORGANIC VS. MINERAL INPUT EFFECTS ON TOTAL PROTEIN CONTENT IN SUMMER OF SPONTANEOUS FLORA OF A HILL PERMANENT GRASSLAND FROM BANAT COUNTY

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Abstract

Protein content of grassland forage is one of the most important qualitative parameter monitored quantitatively when is calculate the daily animal ratio for feeding. In this research the aim was to collect data for total protein content of a forage harvested in summer from a permanent grassland situated in a hill region of Banat County, Romania ($45^o12'$N; $21^o60'$E), on Calcic Luvisol, fertilised mineral or organic. Multivariate analysis technique - Principal Components & Classification Analysis (PC&CA) implemented in STATISTICA 10 software was used to analyse the obtained data. As based/active variables were introduced some ecological soil parameters and fertilisation data. The selected soil parameters were: pH (H_2O), total nitrogen content – Ns (%), phosphorus – Pm (ppm) and potassium – Km (ppm) mobile form. As supplementary variables were used total protein content (%) of hill grassland forage harvested in summer (June 2009) and the gravimetric percent of individual participation of some spontaneous plants: *Festuca rupicola*, *Calamagrostis epigejos*, *Trifolium repens* and *Lathyrus pratensis*. The cases were the seven trials of experimental field, one unfertilised and 6 with different substances flows anthropic influenced by mineral or organic (sheep manure) application. The results were analysed by PC&CA via the correlation matrix. Principal components PC1 and PC2 explained more than 85% of process total variance. The correlation coefficients between total protein content (%) of grassland forage against individual participation data for *Trifolium repens* (%) and *Lathyrus pratensis* (%) were positive and high (0,951, respectively 0,924). Mineral fertilisers applied on grassland had a negative effect on total protein content of forage (correlation coefficients between -0.558 to -0.630), while sheep manure management had a high positive influence (0.976).

Key words: total protein content, fertilisation, quality, grassland, multivariate analysis.

In Romania the quality of meat and dairy products from ruminants depend heavily by the grassland forage quality, because grazing and mowing represent inestimable traditional natural resources for ruminants feeding. Marusca T. (2008) considers that grassland offers the possibility to obtain the cheapest forage, and also has numerous benefits on animals' organism health during all grazing periods.

A qualitative production of milk requests in daily diet of cattle minimum 20% crude protein (Ball D.M. *et al*., 2001). The forage harvesting extracts from grassland ecosystem around 20 kg nitrogen for each tonne of dry matter (Samfira I. and Moisuc A., 2007; Moisuc A. and Sarateanu V., 2008). So an output versus inputs equilibrium is required for grassland.

Fertilisation, mineral and/or organic, is the main measure for nutrients input and represents a very complex issue in permanent grassland case because of the high number of perennial plants species with different nutrients requirements (Iacob *et al.*, 2000). The presence of leguminous species in grassland covering herbaceous mixture is recommended also for improve the nitrogen inputs. *Trifolium* spp., for example, is responsible by the fixing symbiotically from the atmosphere for 100 kg to 380 kg nitrogen / ha (Peyraud J.L. *et al.*, 2009). Another objective in the context of agriculture of our days is to increase the nitrogen utilisation by the animals, decreasing the loss through excretions (Hoekstra N.J. *et al.*, 2007).

The stimulation of native perennial species seeds with a high pastoral value, including with high crude protein content, for feeding grassland forage must represent one of the most important objective in a farm management, both agronomic and economic point of view. The aim of this study was to analyse that organic or mineral input had effects on total protein content in summer of forage from Romanian hill permanent grassland.

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MATERIAL AND METHOD

Experimental field localisation and design. The experimental field was a permanent grassland from Romanian Banat, situated near Gradinari village (45°12'N; 21°60'E), organised following the complete randomised block design with five replications. 25 m2 was the surface of each trial.

For this study were selected seven trials, one unfertilised (**U**), three trials mineral fertilised with NPK complex, ammonium nitrate, superphosphate, and potassium salt (**M1**, **M2** and **M3**) and other three trials organic fertilised with fermented sheep manure (**S1**, **S2** and **S3**). For the exclusive mineral fertilised trials were used the following doses:

- **M1** – 100 N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha);
- **M2** – 150 N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha);
- **M3** - (100+100) N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha).

In exclusive organic fertilised trials case was used 20t/ha (**S1**), 40t/ha (**S2**) and respectively 60t/ha (**S3**) sheep manure.

In 2003 begins the fertilisation of hill permanent grassland, mineral yearly and organic at each two years.

Soil and climatic conditions of experimental field. The hill permanent grassland was situated on Calcic Luvisol. The humus content varied between 6.44%–9.04% for selected organic fertilised trials, 6.19%-8.05% in mineral fertilised cases, reported to unfertilised trial with 6.18%.

The clime in this region is temperate continental with Mediterranean influences (Ianoş Gh., 2005). The rainfall in 2009 was around 76 mm in March, 31mm in April, 67 mm in May and 166 mm in June. The annual average temperature was around 12°C in 2008. In 2009 the average temperatures were: -0.4°C in January, 1.0°C in February, 5.8°C in March, 14.5°C in April, 17.2°C in May and 19.5°C in June. The rainfall and temperature data were collected by the Meteorological Station Archive, Oravita, Caras-Severin district.

Statistical analysis. The multivariate analysis technique PC&CA (Principal Components & Classification Analysis) via the correlation matrix was selected for statistical data interpretation, and was computed by STATISTICA VERSION 10. The fertilisation data were used as active variables. Also as active variables were selected the soil parameters: pH (H_2O), total nitrogen content – Ns (%), mobile form of phosphorus – Pm (ppm) and potassium – Km (ppm). The cases were considered the trials of experimental field. The supplementary variables (*) were: total protein content (%) of harvested forage in June 2009, and gravimetric percent of some plants species of hill permanent grassland covering flora: *Fr (*Festuca rupicola*), *Cal (*Calamagrostis epigejos*), *Tr (*Trifolium repens*), and *Lp (*Lathyrus pratensis*).

Chemical determinations. The total protein content of forage harvested in June 2009 from 1 m^2 surface of each trial, at 3 cm above soil, was determined by Kjeldahl method (AOAC method 954.01, Edition 15/1990). Soil parameters were quantified using specific methods: pH (H_2O) in conformity with SR ISO 10390 (1999); soil total nitrogen content by Kjeldahl method; the mobile form of phosphorus and potassium by Egner-Riehm-Domingo method (Stoica E. *et al.*, 1986).

RESULTS AND DISCUSSIONS

The data of experimental field fertilisation for selected seven trials (one unfertilised, three fertilised organic and three mineral) and the results obtained for soil parameters of hill permanent grassland trials were used as active variables of PC&CA.

The experimental values for pH (H_2O) indicated that in climatic conditions of hill permanent grassland of June 2009, the mineral fertilisation acidified the soil 5.50 (M1), 5.51(M2), 5.43 (M3) comparatively with unfertilised trial (5.72), while organic fertiliser in high doses increased: 5.67 (S1), 5.89 (S2) and 6.00 (S3).

The soil total content of nitrogen (Ns) in this period of year was higher for all selected fertilised trials (mineral and organic) reported to U case with unfertilised soil (0.23%): 0.24% (S1), 0.30% (S2), 0.31% (S3), 0.27% (M1), 0.28%(M2), and 0.27% (M3).

The concentrations of soil phosphorus and potassium mobile form were also higher in experimental trials fertilised mineral (54.78 ppm – M1, 50.55 ppm – M2, 52.70 ppm – M3; and 238 ppm – M1, 224 ppm – M2, 232 ppm – M3) or organic with sheep manure (47.65 ppm – S1, 53.93 ppm – S2, 71.53 ppm – S3; and 233 ppm – S1, 230 ppm – S2, 236 ppm – S3), reported to unfertilised trials (43.88 ppm, and respectively 229 ppm).

The contributions (gravimetric percent) of some spontaneous grass of hill permanent grassland covering flora were used as supplementary variables (*).

Festuca rupicola (*) was the dominant grass in this period of the year in all experimental trials cases of studied grassland forages. In unfertilised case U, the *Festuca rupicola* gravimetric percent was 42%. Sheep manure application at each two years reduced the participation of this grass in grassland covering spontaneous flora: 28% (S1), 22% (S2) and 16% (S3). The mineral fertilisers applied yearly increased the gravimetric percent of *Festuca rupicola*: 51% (M1), 46% (M2) and 43% (M3).

Calamagrostis epigejos (*) grass varied between 5% to 13% in grassland spontaneous flora. In unfertilised case the forage had 8%

Calamagrostis epigejos, while the forage of organic and mineral fertilised trials had 5% (S1), 8% (S2), 8% (S3), 11% (M1), 13% (M2), and 12%(M3).

It was selected also gravimetric percent of two perennial leguminous plants as supplementary variables (*): *Trifolium repens* and *Lathyrus pratensis*. In unfertilised case conditions (U) the leguminous perennial plants were present under 0.1%. The same situation was generally for mineral fertilised trials. In organic fertilisation cases, the clime and soil conditions stimulate both leguminous plants growth. For *Trifolium repens*, dominant leguminous plants in grassland forage, the gravimetric percent was 25% (S1), 27% (S2), respectively 38% (S3), while *Lathyrus pratensis* was under 0.1% (S1), 4% (S2) and 6% (S3). *Trifolium repens* is in our days one of perennial leguminous plant recommended in grassland covering flora because of easier management in mixture with other plants, higher palatability than grasses and other herbages, a high nutritional quality for a longer period than grasses and decreasing the nitrate leaching (Peyraud J.L. *et al.*, 2009).

Another supplementary variable used for computed the multivariate analysis technique PC&CA was total protein content (*) of forages harvested in summer of 2009 from all seven selected experimental trials of hill permanent grassland. In unfertilised ecosystem conditions the total protein content of forage was 8.33%. For exclusive mineral fertilisation cases the total protein content of forage varied from 8.1% (M1), to 8.89% (M2) and 9.29% (M3). The organic fertilisation trials forage had 10.68% (S1), 12.88% (S2), and 13.68% (S3) total protein content, the concentration increased with the increasing of applied organic fertiliser.

The PC&CA technique was performed using all established active and supplementary variables of the selected seven cases for analyse that organic or mineral input has effects on total protein content in summer of spontaneous flora of hill permanent grassland from Romanian Banat. For a quality of 100% of PC&CA were obtained a number of six principal components (PC). The eigenvalues for the six principal components and the corresponding total variance (%) are presented in *table 1*. Principal components PC1 and PC2 described around 86% of total variance of the process.

The principal components PC1 and PC2 loadings based on correlation of active and supplementary (*) variables are shown in *table2*.

Table 1

The eigenvalues for the six principal components and the corresponding total variance (%)

PC number	Eigenvalues	Total variance (%)
1	4.63	57.88
2	2.25	28.07
3	0.93	11.67
4	0.10	1.23
5	0.07	0.86
6	0.02	0.29

Table 2

The factor loadings based on correlation of active and supplementary (*) variables

Variables	PC1	PC2
pH (H_2O)	0.981	-0.011
Ns (%)	0.377	-0.833
Pm (ppm)	0.539	-0.814
Km (ppm)	0.301	-0.390
Manure (t/ha)	0.921	-0.340
N (kg/ha)	-0.866	-0.431
P (kg/ha)	-0.879	-0.467
K (kg/ha)	-0.879	-0.467
*Protein (%)	0.866	-0.313
*Fr (%)	-0.921	0.102
*Cal (%)	-0.744	-0.516
*Tr (%)	0.912	-0.185
*Lp (%)	0.852	-0.465

where: Ns (%) – soil total nitrogen content; Pm (ppm) and Km(ppm) – mobile form of phosphorus and potassium content; *Protein (%) – total protein content of forage; *Fr (%), *Cal (%), *Tr (%), *Lp (%) – gravimetric percent in hill grassland forage of *Festuca rupicola*, *Calamagrostis epigejos, Trifolium repens*, and *Lathyrus pratensis.*

Principal component PC1 had the eigenvalue 4.63 and a corresponding total variance of 57.88 (%). A high positive impact in first principal component structure had the soil pH(H_2O), sheep manure application, grassland forage total protein content (%), and the gravimetric percent in grassland covering flora of perennial leguminous species *Trifolium repens* and *Lathyrus pratensis*. High negative values were obtained for the following variables: gravimetric percent of perennial grass species of hill grassland forage, *Festuca rupicola* and *Calamagrostis epigejos*, and the mineral fertilisation data.

The second principal component (PC2) explained more than 28% of process total variance and had an eigenvalue equal with 2.25. A high negative impact in structure of PC2 had two soil parameters: Ns (%) – total nitrogen content and Pm (ppm) – mobile form of phosphorus.

The projection of the seven selected cases of the hill permanent grassland, unfertilised and with organic or mineral fertilisers management, and the projection of the active variables and supplementary (*) variables on PC1xPC2 plane are shown in *figure 1*, respectively in *figure 2*.

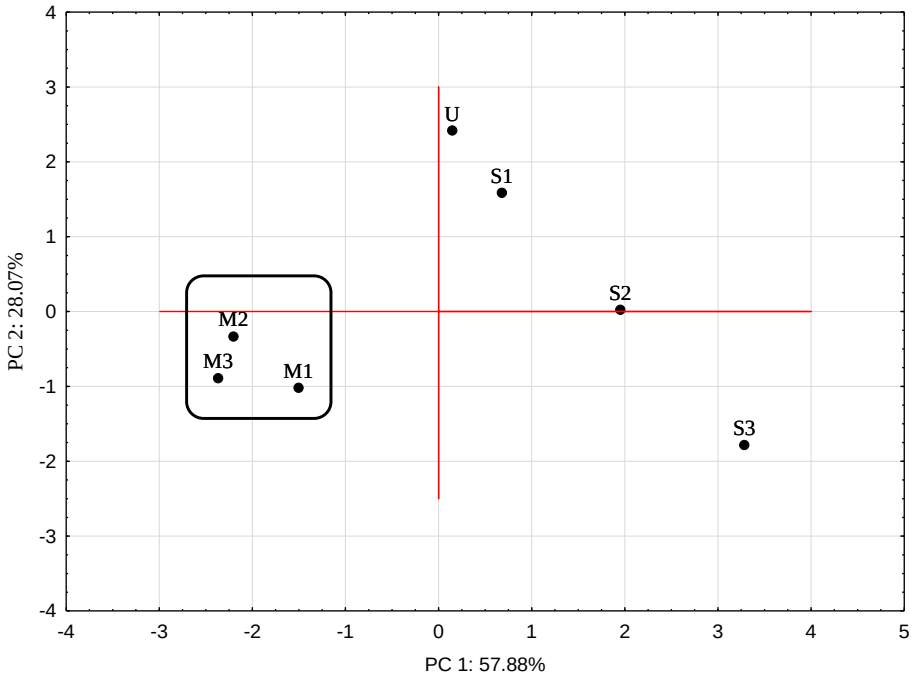

Figure 1 **The projection of the seven selected trials of hill permanent grassland on PC1xPC2 plane**

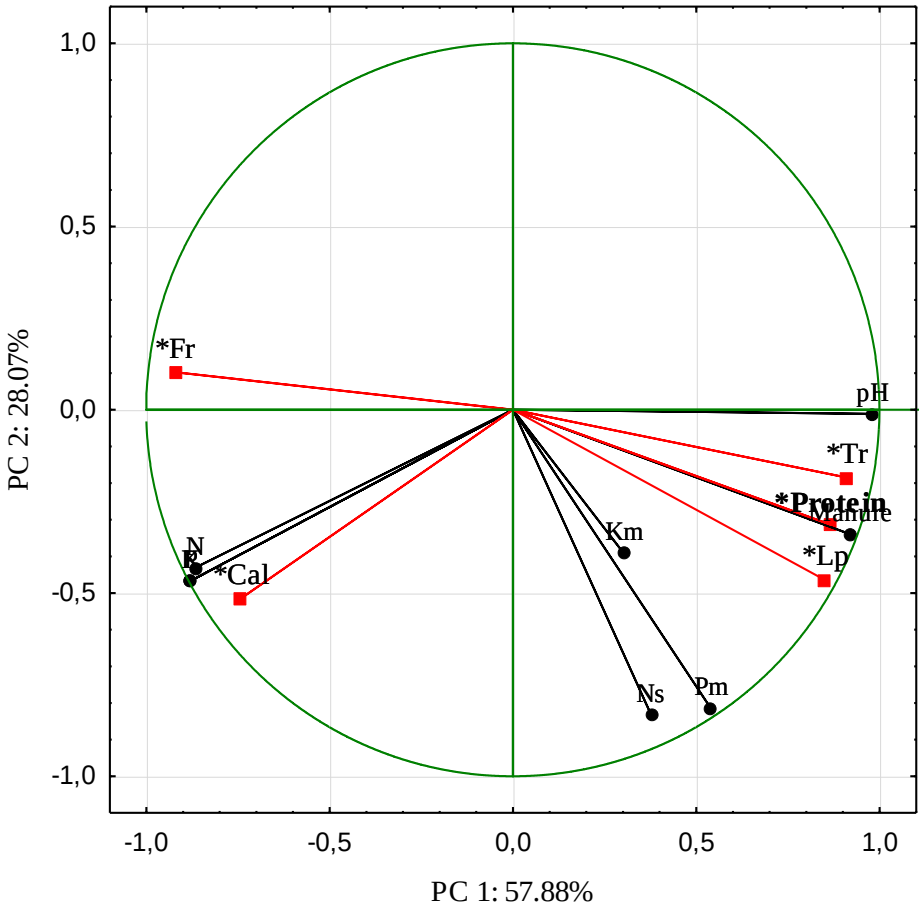

Figure 2 **The projection of the active and supplementary (*) variables on PC1xPC2 plane**

From *figure 1* it can observe that the exclusive mineral fertilisation management in all three cases classified the trials M1, M2 and M3 in the same group. The unfertilised trial (U) and the other three trials with a sheep manure management (S1, S2 and S3) were classified different by the trials with exclusive mineral input using PC&CA.

From *figure 2* and the results of *table 3* (the PC&CA of all variables correlations), it can observe that in June 2009, in soil and clime conditions of hill permanent grassland, the total protein content of forage from sheep manure fertilised trials is done preponderant by the leguminous perennial species *Trifolium repens* (dominant) and *Lathyrus pratensis*.

Table 3

The PC&CA active and supplementary (*) variables correlations

Variables	*Protein (%)	*Fr (%)	*Cal (%)	*Tr (%)	*Lp (%)
pH (H_2O)	0.862	-0.892	-0.643	0.857	0.882
Ns (%)	0.693	-0.478	0.259	0.503	0.786
Pm (ppm)	0.680	-0.559	0.019	0.624	0.824
Km (ppm)	0.152	-0.186	-0.286	0.323	0.261
Manure (t/ha)	0.976	-0.952	-0.523	0.961	0.955
N (kg/ha)	-0.558	0.683	0.884	-0.684	-0.502
P (kg/ha)	-0.630	0.788	0.903	-0.727	-0.538
K (kg/ha)	-0.630	0.788	0.903	-0.727	-0.538
*Protein (%)	-	-0.960	-0.481	0.951	0.924

where: Ns (%) – soil total nitrogen content; Pm (ppm) and Km (ppm) – mobile form of phosphorus and potassium; *Protein (%) – total protein content of forage; *Fr (%), *Cal (%), *Tr (%), *Lp (%) – gravimetric percent in hill grassland forage of *Festuca rupicola*, *Calamagrostis epigejos, Trifolium repens*, and *Lathyrus pratensis.*

The correlation coefficient between total protein content (%) of forage and organic fertilisation management data was high and positive (0.976). The gravimetric percent of *Trifolium repens* and *Lathyrus pratensis* versus forage total protein content had also high positive correlation coefficients: 0.951 and 0.924.

In forage case resulted after an exclusive mineral fertilisation management, the grasses *Festuca rupicola* and *Calamagrostis epigejos* had the highest contribution in covering biomass: 62% (M1), 59% (M2) and 55% (M3). The correlation coefficients between mineral fertilisation data versus total protein content (%) of forage were negatively (from -0.558 to -0.630).

The correlation of mineral fertilisation data against gravimetric percent of the *Calamagrostis epigejos* contribution in grassland forage were positively (from 0.884 to 0.903) and higher than against *Festuca rupicola* gravimetric percent (from 0.683 to 0.788). The supplementary variable total protein content of grassland forage versus *Festuca rupicola* gravimetric percent had a high negative coefficient (-0.960).

The fertilisers management affect the soil pH (H_2O) and directly the stimulation of leguminous (sheep manure) or grasses (mineral fertilisers) perennial species of grassland covering flora. The correlation coefficients were high and positively for the soil pH (H_2O) versus gravimetric percent of *Trifolium repens* and *Lathyrus pratensis* (0.857 and 0.882), while in grasses *Festuca rupicola* and *Calamagrostis epigejos* cases were negatively (-0.892 and -0.643).

CONCLUSIONS

Based on obtained experimental results, it can conclude that in soil and clime conditions of summer 2009 from Romanian Banat, the adopted sheep manure management for all three trials of hill permanent grassland improve significant the total protein content of forage by increasing the leguminous participation in covering spontaneous flora, reported to unfertilised trial. Mineral fertilisers management acidified the soil pH (H_2O) and stimulated the grass perennial species growing, determined a lower total protein content of forage comparatively with organic management cases.

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BD/CNCSIS Romania (www.cncsis.ro), 2007 – 2009.

REFERENCES

Ball D.M., Collins M., Lacefield G.D., Martin N.P., Mertens D.A., Olson K.E., Putnam D.H., Undersander D.J., Wolf M.W., 2001 - *Understanding Forage Quality*. American Farm Bureau Federation Publication 1-01, Park Ridge, IL:1-17.

Hoekstra N.J., Schulte R.P.O., Struik P.C., Lantinga E.A., 2007 - *Pathways to improving the N efficiency of grazing bovines*. Europ. J. Agronomy, 26: 363–374.

Iacob T., Vintu V., Samuil C., 2000 – *Tehnologia producerii si conservarii furajelor*. Edited by Ion Ionescu de la Brad Publisher, Iasi.

Ianos Gh., **2005** - *Geografia solurilor cu notiuni generale de pedologie*. Edited by Universitatea de Vest, Timisoara.

Marusca T., 2008 – Reconstructia ecologica a pajistilor degradate. Edited by Universitatea Transilvania, Brasov.

Moisuc A., Sarateanu V., 2008 – Gazonul. Agroprint Publisher, Timisoara.

Peyraud J.L., Le Gall A., Lüscher A., 2009 - *Potential food production from forage legume-based-systems in Europe: an overview.* Irish Journal of Agricultural and Food Research, 48: 115–135.

Samfira I., Moisuc A., 2007 - *Ecopratotehnica*. EUROBIT Publisher, Timisoara.

Stoica Elena coord., Rauta C., Florea N., 1986 - *Metode de analiza chimica a solului*. Bucuresti.

***** JAOAC *Official Methods of Analysis,* 1990 (954.01)** – *Protein (Crude) in Animal Feed. Kjeldahl method*. edited by Herlich Kenneth, 15th Edition, published by Association of Official Analytical Chemists, Arlington, Virginia, SUA.

***** SR ISO 10390, 1999** - *Calitatea solului. Determinarea pH-ului.*

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